

Article

Changes in Nutrient Biomarkers, Body Composition, and Burnout Symptoms in Collegiate Athletes across a Season

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Abstract: Adequate serum vitamin D and iron levels are thought to positively influence physical training adaptations and mood. The purpose of this prospective, observational, study was to investigate relationships between serum 25-OH vitamin D and serum ferritin levels with body composition and athlete burnout symptoms. Seventy-three collegiate athletes (49 female) from 7 indoor and outdoor sports were tested pre-season and post-season for: nutrient biomarkers (serum 25-OH vitamin D and serum ferritin) via venipuncture; body composition (total lean mass, bone mineral density/BMD, and % body fat) via dual energy x-ray absorptiometry (DXA) scans; and athlete burnout symptoms (post-season) via the athlete burnout questionnaire (ABQ). When male and female cohorts were combined, significant relationships were noted between pre-season serum 25-OH vitamin D versus the change (Δ : post-season minus pre-season) in both BMD ($r=-0.34$; $p=0.0003$) and % body fat ($r=-0.28$; $p=0.015$). Serum ferritin Δ was significantly associated with lean mass Δ ($r=-0.34$; $p=0.003$). For burnout symptoms, serum 25-OH vitamin D Δ significantly explained 20.6% of the variance for devaluation of sport in the male cohort only. Across time, serum 25-OH vitamin D levels increased while serum ferritin levels decreased, non-significantly, in both males and females. Relationships between nutrient biomarkers and body composition were opposite of physiological expectations.

Keywords: vitamin D, ferritin, student-athletes, DXA scan

1. Introduction

The trend towards precision medicine [1] has fueled a more individualized approach towards precision athletic health and training. Assessing nutrient biomarkers, as a potential driver of favorable adaptations in muscle strength [2], performance [3], and mood [4], has fast become standardized practice in elite athlete screenings [5-7]. However, the development of normative values which promote health and peak performance, in the context of both sex and sport, remain to be elucidated.

Blood nutrient biomarkers which reflect vitamin D [6,8-13] and iron [3,5,7,14-16] status are commonly measured in athletes, particularly collegiate athletes [6,7,9-12,14]. Vitamin D (typically assessed as serum 25-OH vitamin D measurement) has emerged as a potential ergogenic aid [2], reducing muscle damage [17], stress fractures [18], and frequent illness [13]. Similarly, correction of low iron levels (typically assessed as serum ferritin measurement) has been shown to improve endurance performance [3], subjective measures of performance [19], and fatigue [16] in female athletes. The downside of blood

biomarker measurement, however, is cost. For example, the average annual cost to screen athletes at one National Collegiate Athletic Association Division 1 (NCAA D1) Institution for serum 25-OH vitamin D was estimated at \$7250 (average cost \$51-100 per laboratory draw) [6] while the estimated cost for serum ferritin measurement was \$4248 (\$32 per laboratory draw) [7].

Another commonly utilized training outcome measure is body composition. Favorable training adaptations include increasing lean [20] and bone [21] mass while decreasing fat mass [20] across a competitive season. Unhealthy adaptations in response to over-training, inadequate nutrition, or relative energy deficiency in sport (RED-S), often manifest as decreased lean [22] or bone [23] mass which, in turn, precedes injury and illness [22-25]. Favorable relationships between nutrient blood biomarkers (such as vitamin D and iron) and body composition variables would thereby underscore the importance of nutrient screening and supplementation, in collegiate athletes, in future.

In addition to markers of physical health, relationships between serum 25-OH-vitamin D and ferritin on athlete mental health remain underexplored. Evidence suggests that low serum 25-OH vitamin D and ferritin levels are inversely related to depression scores [26,27] in university students. However, relationships between serum 25-OH vitamin D and ferritin with burnout symptoms in athletes have yet to be examined.

Therefore, the primary aim of this preliminary investigation was to prospectively investigate (pre- and post-season) relationships between serum 25-OH vitamin D and serum ferritin levels with body composition variables in a cohort of indoor and outdoor NCAA D1 collegiate athletes. A secondary aim was to explore relationships between serum 25-OH vitamin D and ferritin levels with athletic burnout symptoms.

2. Materials and Methods

Student-athletes from a midwestern National Collegiate Athletic Association Division 1 (NCAA D1) University (latitude 42.7°N) were recruited to participate in this prospective, observational, study. Participants from seven different fall and winter sports teams (men and women's cross country/XC, swimming, and basketball plus women's soccer) were recruited and signed written informed consent to participate in this Institutional Review Board (IRB) approved study (Oakland University IRB#778936). Athletes presented to the lab both pre-season (September) and post-season (men and women's XC in December; women's soccer in January; men and women's basketball and swimming teams in April). Athletes were asked to refrain from exercise and eating (other than water, when thirsty) for 4-hours prior to each laboratory visit. Athletes were also instructed to wear compression shorts (plus sport bras for females), without any metal, for the dual energy x-ray absorptiometry (DXA) scans. The following procedures were performed:

2.1 Blood biomarkers: Serum 25-OH vitamin D and serum ferritin were assessed by a local hospital laboratory (Cobas E Immunoassay, Ascension-Crittenton Hospital, Rochester, Michigan), following venipuncture in a supine position. All separated serum specimens were sent to the hospital laboratory within 12-hours of venipuncture. The diagnostic threshold for serum 25-OH vitamin D insufficiency was $\leq 30\text{ng/mL}$ [8,28] while serum ferritin insufficiency was defined as any value $\leq 35\text{ng/mL}$ [29].

2.2 Total body composition: Height and weight were measured in compression clothes, without shoes or socks, on a stadiometer with a weight beam scale. Total body lean, fat and bone mass were measured using a whole-body DXA scan (Horizon A, software version 13.6.0.5, TBAR102 NHANES BCA calibration, Hologic™, Bedford, MA). All DXA scans were performed following standardized protocols, outlined in the product user manual [30]. Athletes who were taller than the scan table parameters were positioned with their feet outside of the scan area, both times (standardized). We chose to report total bone mass as bone mineral density (BMD in g/cm^2) and total fat mass as a percentage (body fat %) to standardize these variables across sex, sport, and body weight. However, we reported total lean mass in kilograms to reflect size and metabolic demand differences.

2.3 Post-season burnout assessment: A 15-question athlete burnout questionnaire (ABQ) was completed, in the laboratory, at the post-competitive season timepoint only [31]. Each question was measured on a 5-point Likert scale, with 1 representing “almost never” and 5 representing “almost always”. Subscales of the ABQ include devaluation of sport, emotional and physical exhaustion, and reduced sense of accomplishment which have been previously validated in a cohort of collegiate athletes [31].

2.4 Statistical analyses: Paired t-tests were utilized to compare pre-season to post-season changes in anthropometric, body composition and blood biomarker variables. Repeated-measures ANOVA analyses were utilized to assess time (pre- to post-season) and team (sports) differences, plus possible interactions between time vs. team (F-value). Linear relationships between variables were assessed using Pearson’s *r* for continuous variables (blood biomarkers and body composition) and Spearman’s rank-order correlations for categorical variables (ABQ). The effect size for each correlation coefficient (*r*) was also interpreted using Cohen’s *d* whereas: *r* = 0.2 represented a small effect; *r* = 0.5 represented a medium effect; and *r* = 0.8 represented a large effect [32].

The pre-season to post-season change for all continuous variables were denoted by the symbol “ Δ ”, which reflected the mathematical calculation of “post-season minus pre-season”. Therefore, any positive Δ value represented an increase while any negative Δ value represented a decrease in the stated variable across time. All data expressed as mean \pm SD, with statistical significance set *a priori* at *p* < 0.05.

3. Results

Seventy-three student-athletes (49 female) completed both pre-season and post-season testing. Pre-season demographic and anthropometric data for both male and female cohorts, separated further into sports teams, are reported in Table 1.

Table 1: Student-athlete demographics by sex and sports team (XC = cross country; BMI=body mass index). All data reported as means \pm SD. ♀=female; ♂=male.

Team (N=73)	Age (years)	Height (m)	Weight (kg)	BMI (kg/m ²)
♀ XC (n=16)	19.4 \pm 1.5	1.64 \pm 0.1	56.6 \pm 4.9	20.8 \pm 1.8
♂ XC (n=9)	20.3 \pm 0.9	1.80 \pm 0.1	68.8 \pm 6.9	21.3 \pm 0.9
♀ Swim (n=12)	19.6 \pm 1.1	1.70 \pm 0.1	67.2 \pm 5.8	23.2 \pm 2.1
♂ Swim (n=6)	20.7 \pm 1.2	1.84 \pm 0.1	78.5 \pm 5.2	23.3 \pm 1.6
♀ Basketball (n=10)	19.7 \pm 1.4	1.76 \pm 0.1	75.1 \pm 12.0	24.3 \pm 3.4
♂ Basketball (n=9)	20.1 \pm 1.5	1.96 \pm 0.1	94.5 \pm 14.3	24.6 \pm 2.8
♀ Soccer (n=11)	19.5 \pm 1.2	1.69 \pm 0.1	64.9 \pm 14.4	22.6 \pm 3.6
♀ COMBINED (n=49)	19.5 \pm 1.3	1.69 \pm 0.1	64.8 \pm 11.5	22.5 \pm 2.0
♂ COMBINED (n=24)	20.3 \pm 0.1	1.87 \pm 0.1	80.9 \pm 15.0	23.0 \pm 2.4

The pre-season and Δ in body composition variables for both male and female cohorts, separated further by sports team, are reported in Table 2. No statistically significant differences (pre-season vs. post-season; paired t-test analyses) were noted for any body composition measure across time for any team. Repeated measures ANOVA analyses did

not detect any significant interactions between time vs. team for any body composition variable.

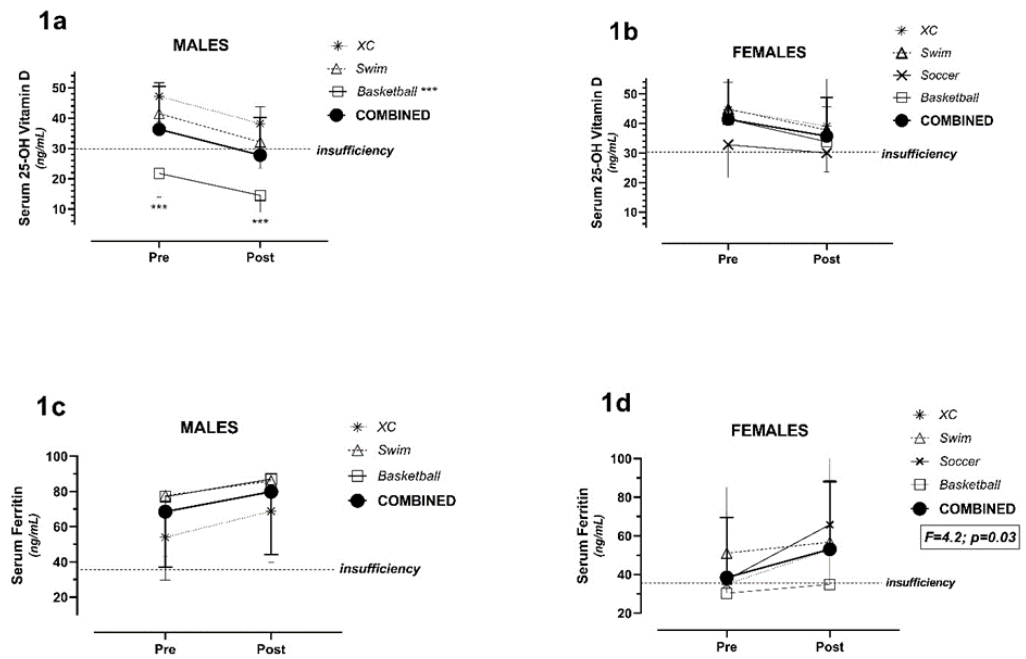
Table 2: Pre-season and change (Δ : post-season minus pre-season) in total body fat (%), bone mineral density (BMD), and lean mass in student-athletes by sex and sports team (XC = cross country. BMD = bone mineral density). All data reported as means \pm SD. ♀=female; ♂=male

Team (N=73)	Pre Body Fat (%)	Δ Body Fat (%)	Pre BMD (g/cm ²)	Δ BMD (g/cm ²)	Pre Lean Mass (kg)	Δ Lean Mass (kg)
♀ XC (n=16)	18.7 \pm 3.1	-0.8 \pm 1.1	1.08 \pm 0.06	-0.00 \pm 0.02	43.7 \pm 4.3	-0.4 \pm 0.9
♂ XC (n=9)	8.5 \pm 1.1	-0.1 \pm 0.8	1.22 \pm 0.05	-0.01 \pm 0.01	59.4 \pm 6.1	-0.3 \pm 0.9
♀ Swim (n=12)	21.2 \pm 3.8	-1.7 \pm 1.8	1.08 \pm 0.07	0.00 \pm 0.02	49.0 \pm 3.0	1.8 \pm 1.3
♂ Swim (n=6)	12.0 \pm 2.4	-1.3 \pm 1.7	1.17 \pm 0.07	0.00 \pm 0.01	66.0 \pm 5.5	0.5 \pm 2.1
♀ Basketball (n=10)	18.2 \pm 5.1	-0.7 \pm 1.1	1.19 \pm 0.09	0.01 \pm 0.02	56.1 \pm 5.9	-0.6 \pm 1.9
♂ Basketball (n=9)	10.7 \pm 3.0	0.8 \pm 1.4	1.37 \pm 0.09	0.02 \pm 0.02	77.8 \pm 9.1	-1.3 \pm 1.4
♀ Soccer (n=11)	19.9 \pm 5.0	0.2 \pm 1.5	1.09 \pm 0.07	0.01 \pm 0.01	48.7 \pm 6.5	-0.8 \pm 1.5
♀ COMBINED (n=49)	19.5 \pm 4.2	-0.8 \pm 1.5	1.10 \pm 0.08	0.00 \pm 0.02	48.7 \pm 6.6	-0.2 \pm 1.6
♂ COMBINED (n=24)	10.2 \pm 2.6	-0.1 \pm 1.5	1.27 \pm 0.11	0.01 \pm 0.02	67.9 \pm 10.8	0.3 \pm 3.8

Pre-season and post-season biomarker measurements for serum 25-OH-vitamin D in males (1a) and females (1b) and serum ferritin concentrations in males (1c) and females (1d) are depicted in Figure 1. No statistically significant differences (pre-season vs. post-season; paired t-test analyses) were noted for any biomarker measure for any team. Repeated measures ANOVA analyses detected a significant interaction between time vs. team for serum ferritin in the female cohort only ($F=4.2$; $p=0.03$).

With specific regards to biomarker changes in male participants, the mean serum 25-OH vitamin D levels in the male basketball team was significantly lower than the mean value for male cross country (XC) and swim teams both pre- and post-season (Figure 1a). Thirty-eight percent of all male athletes were 25-OH vitamin D insufficient pre-season increasing to 50% at post-season. Two male athletes (8%) were diagnosed with serum ferritin insufficiency pre-season, while only one male athlete demonstrated serum ferritin insufficiency post-season (4%).

With specific regards to biomarker changes in female participants, no significant time or team differences in mean 25-OH vitamin D levels were observed (Figure 1b). Twenty-four percent of female athletes were 25-OH vitamin D insufficient pre-season increasing to 35% at post-season. A significant interaction effect (time versus team) was noted for serum ferritin for females, with the female soccer players demonstrating lower pre- to post-season changes compared with the XC, swim, and basketball teams (Figure 1d). Fifty-seven percent of all female participants were diagnosed with serum ferritin insufficiency pre-season which improved to 31% post-season.



*** $p<0.001$ between Basketball vs. XC and Swim, both pre-season and post-season (Figure 1a) $p<0.05$ between Soccer vs. Swim and Basketball for Δ serum ferritin (Figure 1d)

Figure 1: Pre-season to post-season changes in serum 25-OH Vitamin D and serum ferritin concentrations by sex and sports team.

With regards to correlation analyses, when both male and female student-athletes were combined ($N=73$), significant negative correlations were observed between pre-season 25-OH vitamin D levels with both BMD Δ (Figure 2a) and % body fat Δ (Figure 2b). Serum ferritin Δ was also inversely related with lean mass Δ , across the competitive season (Figure 2c). All three correlation coefficients ($\sim r = 0.3$) suggests that the effect sizes of these relationships were small, despite meeting the criteria for statistical significance [32]. No other significant correlations were noted between serum 25-OH-D Δ vs. lean mass Δ ($r=-0.00$; $p=0.94$), BMD Δ ($r=0.04$; $p=0.74$) or % body fat Δ ($r=-0.03$ $p=0.80$) or between serum ferritin Δ vs. BMD Δ ($r=0.06$; $p=0.63$) or % body fat Δ ($r=0.17$; $p=0.13$).

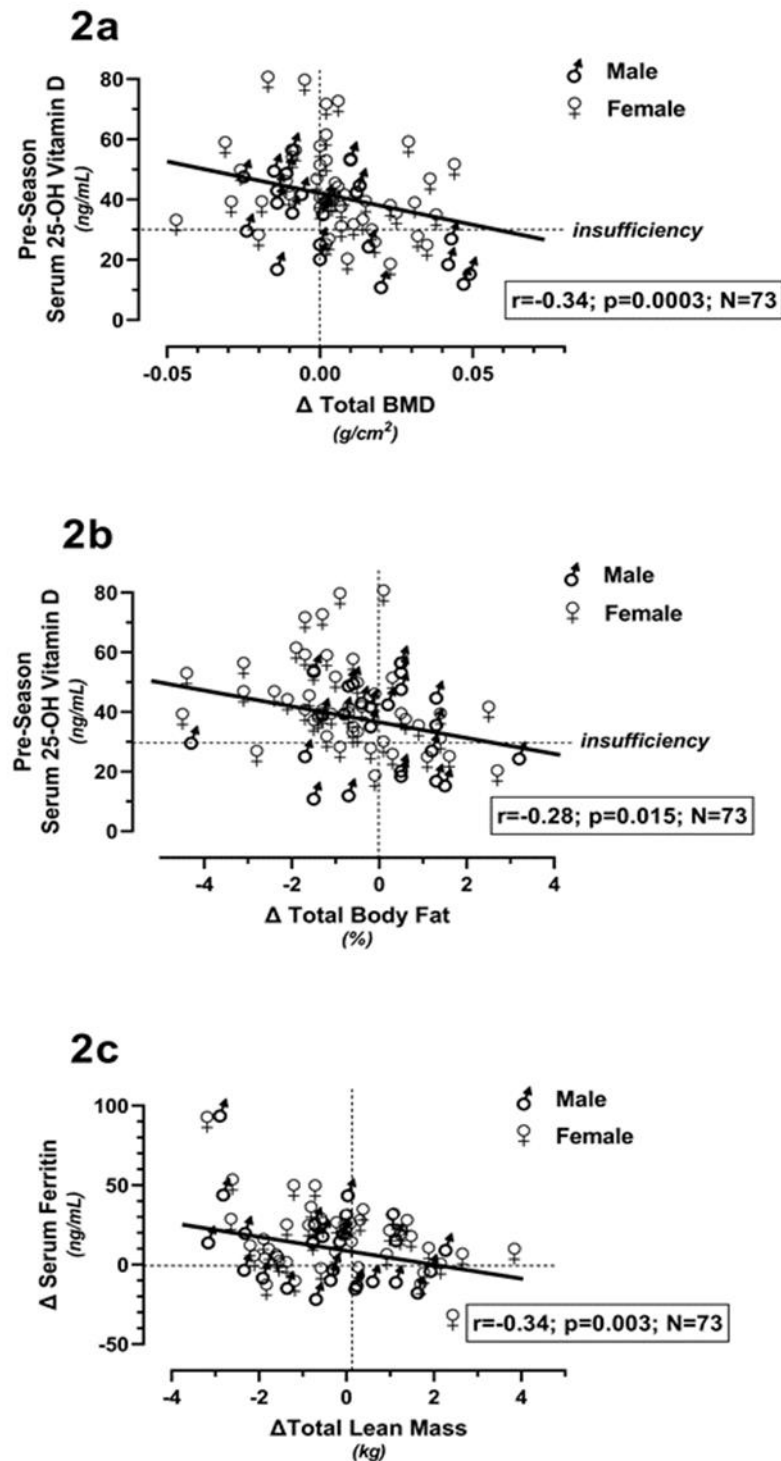


Figure 2: Statistically significant correlations between pre-season serum 25-OH-Vitamin D and changes (Δ : post- minus pre-season change) in bone mineral density (BMD)(2a) and total body fat % (4b) as well as between changes in serum ferritin and changes in total body lean mass (2c) when all athletes were combined ($N=73$).

When the male cohort ($n=24$) was analyzed separately, stronger negative correlations were noted between pre-season 25-OH vitamin D versus all three major body composition variables, pre-season (Figure 3a, 3b, and 3c) and with BMD Δ (Figure 3d). The correlation coefficients suggested a moderate effect [32] among 25-OH vitamin D levels versus body composition variables. Of particular note, male basketball players paradoxically

demonstrated the lowest 25-OH vitamin D blood biomarker levels, of all athletes tested, but had the most favorable body composition parameters recorded (i.e. highest BMD and lean mass, with the lowest body fat percentage).

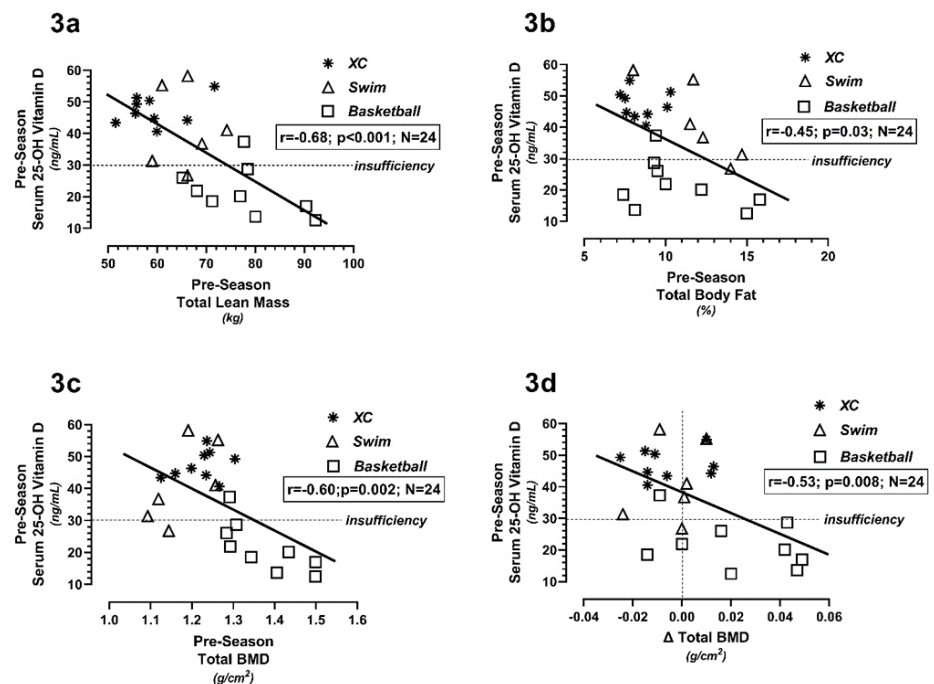


Figure 3: Statistically significant correlations between pre-season 25-OH-Vitamin D and pre-season total body lean mass (3a), body fat % (3b), bone mineral density (BMD)(3c), and changes in BMD (3d) for the male cohort only ($n=24$).

When the female cohort ($n=49$) was analyzed separately, serum ferritin Δ was negatively correlated with lean mass Δ (Figure 4a) and positively correlated with % body fat Δ (Figure 4b). The correlation coefficients suggested small-to-moderate effect sizes [32].

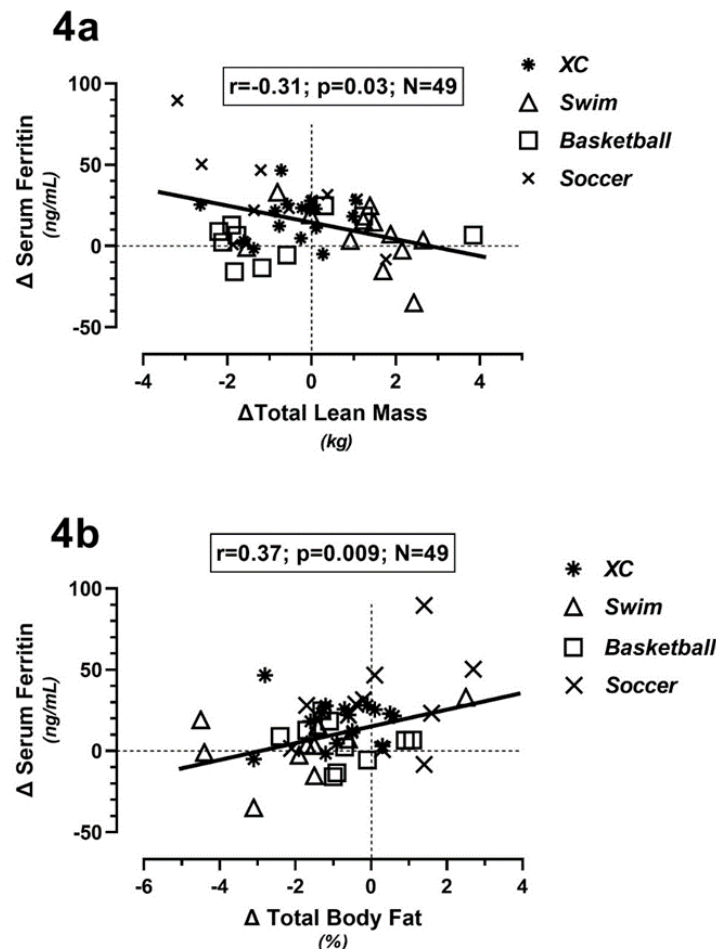


Figure 4: Statistically significant correlations between changes (Δ : post- minus pre-season change) in serum ferritin concentration and changes in total body lean mass (4a) and changes in total body fat % (4b) for the female cohort only ($n=49$).

For the athlete burnout symptoms analyses, we conducted three linear regression analyses using the entire cohort ($N=72$; one athlete did not complete the post-season ABQ). None of the three equations were significant, using either serum 25-OH vitamin D Δ ($p = 0.78$) or serum ferritin Δ ($p = 0.69$) to predict each ABQ subscale. When analyses were separated by sex, neither serum 25-OH vitamin D Δ ($p = 0.57$) nor serum ferritin Δ ($p = .57$) predicted athlete burnout symptoms in females.

However, a significant decrease in serum 25-OH vitamin D Δ ($-8.4 \pm 4.3\text{ng/mL}$) did significantly predict and explain 20.6% of the variance of male athlete perceptions of devaluation of sport ($p < 0.05$). Additionally, pre-season serum ferritin levels ($68.5 \pm 31.4\text{ng/mL}$) explained 16% of the variance of male athlete perceptions of physical and emotional exhaustion from sport ($p \leq 0.05$). Additionally, pre-season serum ferritin and athlete burnout ($r = .41; p < 0.05$), post-season serum ferritin levels and athlete burnout ($r = .36; \text{NS}$), and 25-OH vitamin D Δ and athlete burnout ($r = .41; p < 0.05$) were significant.

4. Discussion

Serum 25-OH vitamin D levels decreased in our cohort during the competitive season, for both male and female student-athletes, in both indoor (swimming, basketball) and outdoor (XC and soccer) sports (Figure 1a and 1b). A similar seasonal decline was documented in 47 indoor and outdoor University athletes, living in the United Kingdom (51.2°N), tested in Fall (pre-season) and Spring (post-season) [33]. Although serum 25-OH vitamin D Δ was not statistically significant in the overall cohort in the present study, post-

season 25-OH vitamin D levels were lower in male vs. female athletes ($27.8 \pm 12.4\text{ng/mL}$ vs. $35.7 \pm 13.1\text{ng/mL}$; $p=0.01$). This sex difference has been verified in NCAA D1 athletes tested elsewhere [9] and of unclear biological or performance significance.

The low serum 25-OH vitamin D levels seen in our male athletes were largely driven by the male basketball players, all of which were classified as vitamin D insufficient post-season (Figure 1a). Low 25-OH vitamin D levels in male basketball players has been verified in the literature [8,10,34], with dark skin color [9,10,35] and limited sunlight exposure [36,37] being the most biologically plausible explanation for this sports-specific finding.

Paradoxically, those student-athletes with the lowest serum 25-OH vitamin D levels demonstrated the largest BMD increases across the season (Figure 2a). This significant inverse relationship between pre-season 25-OH vitamin D and BMD Δ was physiologically unexpected, given the interactive role vitamin D plays in enabling calcium mobilization and subsequent bone mineralization [28,38]. However, an inverse relationship between 25-OH vitamin D versus BMD has been documented previously in collegiate basketball players [10], which suggests that physical training may play a larger stimulatory role in bone formation [21] than isolated serum 25-OH vitamin D levels which warrants further scientific and temporal clarification.

Our investigation also demonstrated a significant inverse relationship between pre-season 25-OH vitamin D and % body fat Δ (Figure 2b). This negative relationship has been previously documented in a cross-sectional investigation of 42 collegiate athletes tested in the Fall [39]. In that study, the authors hypothesized that increased fat stores sequestered vitamin D away from the circulation [39]. Although vitamin D is a fat soluble vitamin, other cross-sectional studies performed on collegiate female indoor athletes [11], or a longitudinal (pre- to post) study performed on collegiate basketball players, found no significant correlations between 25-OH vitamin D and body fat [10].

Of note, significant relationships between serum 25-OH vitamin D and body composition variables were largely present in our male, but not female, cohort (Figure 3). Fifty percent of male athletes and 35% of female athletes, in the present study, were vitamin D insufficient post-season. No significant relationships were noted between any body composition variable and serum 25-OH vitamin D in our female cohort. This lack of relationship between body composition and serum 25-OH levels in females has been demonstrated previously in a cohort of 36 collegiate female athletes [11] and of unclear physiological relevance.

The male cross country (XC) runners in the current study, tended to have the highest serum 25-OH vitamin D levels, presumably from enhanced sunlight exposure during outdoor training and competition compared with indoor sport athletes such as swimmers and basketball players [13]. However, the higher absolute serum 25-OH vitamin D levels were not associated with higher BMD (Figure 3c and 3d), lean mass (Figure 3a), or fat percentage (Figure 3b) compared with swimmers or basketball players. These cross-sectional relationships highlight the complexity of assigning “peak” performance serum 25-OH vitamin D thresholds, such as the 40ng/mL threshold proposed for athletes [2], across a variety of sports. For example, male basketball players may function optimally within the $12\text{-}20\text{ng/mL}$ normal range threshold recommended for bone health by the National Academy of Medicine [40] while other athletes may not [2]. Although screening for serum 25-OH vitamin D remains popularized in elite athletes [6,8,12], evidence to support general population screening for vitamin D deficiency remains inconclusive [41].

In contrast to the pre- to post-season decreases in serum 25-OH vitamin D seen in the present study, increases in serum ferritin levels were seen across the season for all sports teams. Serum ferritin levels were significantly lower in female vs. male athletes both pre-season (38.5 ± 3.0 vs. $68.5 \pm 31.5\text{ng/mL}$; $p=0.0002$) and post-season (53.1 ± 35.0 vs. $79.9 \pm 35.8\text{ng/mL}$; $p=0.003$), due to increased blood losses (menses) in pre-menopausal females [3,42]. Student-athletes meeting the threshold for serum ferritin insufficiency ($\leq 35\text{ng/mL}$) pre-season (57% of females and 8% of males) were counseled by the team nutritionist to increase iron ingestion through diet and self-supplementation as supplements were not provided (or prescribed) by the Athletic department. With that said, it was interesting to

note that serum ferritin levels increased across time in the male cohort who were not counseled to increase iron intake. This unexpected increase in serum ferritin across both time and sport highlights the complexity of utilizing serum ferritin as a singular marker of tissue (mainly liver) iron stores, as serum ferritin is also an acute phase inflammatory marker [43]. Thus, increases in serum ferritin, as a measure of tissue iron stores across a competitive season, may be confounded by training or competition-induced inflammation as previously verified in athletes after endurance races [29,44]. The contributions of inflammation to increased serum ferritin levels (non-related to iron storage levels) thereby warrants further investigation using additional inflammatory markers such as hepcidin [3], C-reactive protein and interleukin-6 [44] in future athlete studies.

Serum ferritin Δ levels were inversely related to lean mass Δ , when all athletes were combined (Figure 2c). The negative direction of this relationship suggests that those athletes who gained the most lean mass over the season demonstrated the steepest declines in serum ferritin levels. A 12-week strength training intervention performed on 40 non-athlete college students demonstrated similar increases in fat-free mass, with decreases in serum ferritin levels, in male subjects who started the training regimen with normal serum ferritin levels [45]. The authors of that training study suggested that the increase in training depleted iron stores, although this finding did not apply to their female athletes or participants (male or female) with low baseline serum ferritin levels [45].

Unlike serum 25-OH vitamin D, only the female athletes in the present study demonstrated significant relationships between serum ferritin Δ versus body composition variables (Figure 4). The directionality of these associations was also paradoxical, as increased serum ferritin levels across the competitive season were associated with decreased lean mass (Figure 4a) and increased body fat percent (Figure 4b). Decreased muscle mass and increased fat usually represent unfavorable training adaptations which signify overtraining, undertraining, under-recovery, or even under-nutrition with or without RED-S [20,22-24]. Although published studies clearly suggest that increased serum ferritin levels (from iron supplementation) enhance both endurance performance [3,16] and strength gains [3], the physiological effects of serum ferritin levels on body composition markers requires more controlled investigations - including dietary and caloric intake data - particularly in female athletes participating in aesthetic and gravitational sports.

Preliminary associations between post-season athlete burnout with either serum 25-OH vitamin D or serum ferritin levels (pre-season, post-season or Δ) were not significantly correlated when all athletes were combined. However, when separated by sex, devaluation of sport (as a subscale) was negatively related to serum 25-OH vitamin D Δ while pre-season serum ferritin was positively related to physical and emotional exhaustion in male athletes only. Previous studies have shown that 5-days of vitamin D3 enhances mood, when compared to placebo supplementation [4]. Similarly, a significant inverse relationship was documented between serum 25-OH vitamin D and depression scores in 615 healthy male and female University students (45.5° S) [26]. Collectively, these studies suggest the possibility of a biopsychological link between mood and vitamin D. With regards to serum ferritin, a cross-sectional study performed on 192 female medical students found that serum ferritin levels were lower in depressed compared with non-depressed students [27]. Thus, a possible interplay between nutrient biochemistry and athlete burnout appears plausible and worthy of more critical follow-up in future, especially amongst male athletes.

Limitations: Changes in body composition (Table 2) and blood nutrient biomarkers (Figure 1) were perhaps too small (i.e. statistically and clinically insignificant) to make meaningful conclusions regarding optimal performance “thresholds” for serum 25-OH vitamin D and ferritin levels in athletes. Athletes with insufficient serum 25-OH vitamin D or ferritin at baseline (pre-season) were encouraged to ingest supplements at the counseling and recommendation of the team nutritionist (CA), but supplementation was never recorded. This lack of dietary evaluation, dietary supplement control, quantification of skin color and/or sunlight exposure compromised our ability to properly interpret the paradoxical relationships demonstrated between serum 25-OH vitamin D and ferritin

markers with body composition changes. The staggered endpoint for each sport (i.e. post-season testing) also offered additional confounding variables in interpreting relationships between nutrient biomarkers and body composition changes. Nonetheless, these preliminary results provide pilot data to launch more carefully controlled - and targeted - studies regarding the predictive utility of serum 25-OH vitamin D and serum ferritin measurement on body composition and mood changes across a longer timeframe.

5. Conclusions

Serum 25-OH vitamin D levels declined in all athletes, regardless of indoor or outdoor sport, across the competitive season. The male athletes had lower 25-OH vitamin D levels and increased burnout symptoms compared with female athletes. Conversely, serum ferritin levels increased across the season in both male and female athletes, with female athletes having lower serum ferritin levels compared with male athletes. The directionality of relationships between 25-OH vitamin D levels and total fat, lean and bone in male athletes were opposite of what was physiologically expected and largely driven by very low 25-OH vitamin D levels in the male basketball players. Similarly, the directionality of relationships between serum ferritin levels and lean mass and fat percentage in female athletes were opposite of expected and paradoxically reflect a deleterious training response. Collectively, these results highlight the difficulties in creating “blanket” threshold recommendations for serum 25-OH vitamin D and ferritin levels to maximize body composition and mood changes across a variety seasons, sex and sports.

Our results also add caution to the utilization of single biomarkers - and nutrients - to predict complex training outcomes favoring peak athlete health and performance. Additionally, the results from the present study question the current trend towards “biomarker creep” in sports performance. For example, the normal cut-off threshold for serum 25-OH vitamin D in healthy individuals is 12ng/mL while a threshold of 40ng/mL is recommended for athletes seeking peak performance [2]. Similarly, the normal cut-off threshold for serum ferritin for healthy individuals is 12ng/mL [19] while thresholds as high as 50ng/mL have been recommended for male endurance athletes [15]. These elevated blood nutrient biomarker recommendations for athletes exist without compelling evidence, from randomized-control trials, which support enhanced physical or mental performance. Thus, elevated “athlete specific” thresholds for both serum 25-OH vitamin D and serum ferritin requires further recalibration.

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Institutional Review Board Statement: The study was conducted according to the guidelines of the Declaration of Helsinki and approved by the Institutional Review Board (or Ethics Committee) of Oakland University (IRB#778936, approval granted August 10, 2016).

Informed Consent Statement: Informed consent was obtained from all subjects involved in the study.

Data Availability Statement: All data will be available on request to the corresponding author (THB).

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